maintaining the contacting cells and composition under conditions and for a time sufficient to cause the cells to grow, without the addition of soluble growth effector molecules.

Remarks

Claims 1-6, 8-22, and 24-33 are pending after entrance of this Amendment. Claims 1, 5, 6, 8, 13, 21, 22, 24, 31, and 32 have been amended, claims 7 and 23 cancelled, and new claim 33 has been added with this Amendment. A copy of all pending claims as they are believed to have been amended is attached to this Amendment and Response in an Appendix.

Amendments to the Claims

The claims have been amended to more clearly define the claimed invention as a substrate having bound thereto an effective concentration of growth factors to stimulate cell growth, wherein the tethers are water soluble, each tether is able to bind more than one growth effector molecule and the cells do not bind to the polymeric tethers. Support for the aspect of water solubility is found in the specification at page 6, line 24- page 7, line 2 and previously pending claim 7. Support for the aspect of the cells not binding to the tethers is found in the specification at page 27, line 27- page 7, line 2. Each of the polymers listed is known to not bind to cells. Support for the aspect of each tether being able to bind more than one growth effector molecule is found in the specification at page 4, lines 17-18, page

7, lines 3-9, and page 12, lines 25-28. Support for the aspect of growth of cells without the addition of soluble growth effector molecules is found on page 15, lines 25-27.

The Drawings

Please see the accompanying letter to the Draftsman regarding the proposed change to Figure 2, which is also shown in red ink on the attached sheet. The word "coupled" has been changed to "tethered".

Rejections under 35 U.S.C. §112

Claims 1-32 were rejected under 35 U.S.C. §112, second paragraph, and claim 8 was rejected under 35 U.S.C. §112, fourth paragraph. Claims 5, 6, 21, and 22 have been amended to clarify that it is the substrate polymer that is meant. Claim 8 has been amended to delete "starch". The rejection as to the use of the term "enhance" in the claims is respectfully traversed. The term is properly defined in the specification and one of skill in the art would be able to determine without undue experimentation what is meant to be claimed as the invention.

Rejections under 35 U.S.C. §102 and/or §103

Clapper

Claims 1-9, 13, 18-25, and 31 were rejected under 35 U.S.C. §102(b) as disclosed by U.S. Patent No. 5,512,424 to Clapper et al. ("Clapper"). Claims 10-12 and 26-28 were rejected under 35 U.S.C. §103(a) over Clapper. This rejection is respectfully traversed if applied to the amended claims.

Clapper describes the use of a combination of a cell adhesion factor and a positively charged molecule, each bound to the surface of a cell culture support, to promote cell adhesion to the support, in order to culture adhesion- or attachment-dependent cells. Clapper teaches that the use of the combination of a positively charged molecule and a cell adhesion factor is advantageous because the cells can attach through both receptor-mediated attachment, i.e. by interaction with the cell adhesion factor, and also non-receptor-mediated, i.e. charge-related adhesion, through interaction of the negatively charged cell constituents and the positively charged molecules. See Clapper column 6, lines 25-31. Clapper encourages cell adhesion to the positively-charged polymers. The positively charged polymers include carboxymethylcellulose, as noted by the Examiner, but it has been modified by the addition of positively charged groups. See Clapper column 7, lines 24-column 8, line 2 and claim 1.

In the claimed compositions and methods, on the other hand, tethers are made out of polymers which are water soluble and which will not bind to the cells. This is desirable in Applicants' compositions and methods to provide the tethered growth factors with flexibility and substantial mobility, a characteristic which is deemed critical in Applicants' compositions and methods. See the specification, page 6, lines 13-26 ("Substantial mobility of a tethered growth factor is critical . . . ."). The claims have been amended to reflect these characteristics of the tether.

Moreover, as argued in the Amendment of January 23, 1997, Clapper teaches the use of cell adhesion factors and not growth effector molecules. The only discussion is of the use of molecules which enhance **attachment**, not cell growth. It is important to read line 16 of col. 7 in context: "a sufficient density of cell adhesion factor should be carded by the bioreactor's supporting surface to promote cell attachment and growth"; this is not enhanced growth, but merely attachment-dependent growth, i.e., if the cells do not attach, they do not grow. See, for example, the discussion at col. 8, lines 54-67 and col. 10, lines 29-39.

As the Examiner recognizes, Clapper does not teach or suggest a desired backbone length for the positively charged polymers. In fact, Clapper does not suggest that backbone length is important in the invention taught therein. The mere statement by the Examiner that backbone length is "an art-recognized, result-effective variable which is routinely determined and optimized in any art involving polymers", is not sufficient to maintain this rejection. The bare assertion that something is a "design choice" is insufficient to establish a suggestion in the art for the claimed invention. See, *e.g.*, *Northern Telecom, Inc. v. Data Point Corp.*, 15 USPQ2d 1321 (Fed. Cir. 1990).

Herweck et al. in view of Merrill

Claims 1-9, 13-16, 18-25 and 31 were rejected under §103 as obvious over U.S. Patent No. 5,370,681 to Herweck et al. ("Herweck"), in combination with U.S. Patent No. 5,171,264 to Merrill ("Merrill '264"). This rejection is respectfully traversed if applied to the amended claims.

As the Examiner notes, Herweck does not teach biocompatible tethers which have one end covalently linked to a substrate and a growth effector molecule covalently linked to the other end. Nor does Herweck contain any suggesting for incorporating such a tether. Merrill '264 discloses immobilized star PEO molecules "that can be used as a tool for separating and purifying biological molecules". There is no suggestion for binding growth effector molecules to the PEO molecules or for using the PEO molecules in cell growth applications. Therefore, there is no motivation to combine the references, as required. "There must be some reason, suggestion, or motivation found in the prior art whereby a person of ordinary skill in the field of the invention would make the combination." *In re Oetiker*, 24 USPQ2d 1443 (Fed. Cir. 1992)

There is no teaching or suggestion in either Herweck or Merrill '264 that would lead one of skill in the art to make at a minimum the following alterations: select bioactive molecules **enhancing** growth rate and the amount required to enhance growth rate when not internalized and to chemically couple the molecules to the substrate in a density and with appropriate linkers to result in enhanced growth rates of attached cells.

Herweck in view of Merrill '264 and Merrill

Claims 10-12 and 26-28 were rejected under §103 as obvious over Herweck in view of Merrill '264 and further in view of Merrill, J. Biomater. Sci. Polymer. 5, 1-11 (1993). This rejection is respectfully traversed if applied to the amended claims.

Merrill also does not contain a suggestion for attaching growth effector molecules to PEO tethers at an amount effective to enhance growth rate of cells when not internalized and to chemically couple the molecules to the substrate in a density and with appropriate linkers to result in enhanced growth rates of attached cells. Therefore, the combination of references is inappropriate. Moreover, even if the references were to be combined, the claimed compositions and methods are not taught.

Herweck in view of Merrill '264 and Mikos

Claim 17 was rejected under §103 as obvious over Herweck, in combination with Merrill '264 in combination with U.S. Patent No. 5,522,895 to Mikos et al. ("Mikos"). This rejection is respectfully traversed if applied to the amended claims. Claim 17 is dependent upon claim 13 which, as discussed above, is not taught or made obvious by Herweck and Merrill '264. Mikos does not add the elements missing from the Herweck/ Merrill combination and this rejection should be withdrawn in view of the allowability of claim 13.

Mikos is similar to Herweck in that it is directed to a matrix for seeding with cells that can be implanted. It also does not disclose or make obvious selecting bioactive molecules **enhancing** growth rate, determining the amount required to enhance growth rate when not internalized, and to chemically couple the molecules to the substrate in a density and with appropriate linkers to result in enhanced growth rates of attached cells.

Herweck in view of Merrill '264 and Naughton

Claims 29 and 32 were rejected under §103 as obvious over Herweck in combination with Merrill '264 and U.S. Patent No. 5,032,508 to Naughton et al. ("Naughton"). This rejection is respectfully traversed if applied to the amended claims.

Naughton is actually quite similar to Clapper. It discloses a matrix which might be suitable for implantation, having attached thereto stromal cells that serve as attachment factors for other types of cells grown on the matrix. One skilled in the art would be led by the disclosure of Naughton to believe that no further modifications were necessary in order to grow cells since the stromal cells result in adequate cell attachment and growth. Moreover, Naughton also does not contain a suggestion for combining the individual teachings of Herweck and Merrill and does not disclose or make obvious selecting bioactive molecules **enhancing** growth rate, determining the amount required to enhance growth rate when not internalized, and to chemically couple the molecules to the substrate in a density and with appropriate linkers to result in enhanced growth rates of attached cells.

Herweck in view of Merrill '264 and Tomomura

Claims 29 and 30 were rejected over Herweck in combination with Merrill '264 and Tomomura et al. J. Cell. Physiol. 30:221-227 (1987). This rejection is respectfully traversed if applied to the amended claims. Tomomura also does not contain a suggestion for combining the individual teachings of Herweck and Merrill and does not disclose or make obvious selecting bioactive molecules **enhancing** growth rate, determining the amount

required to enhance growth rate when not internalized, and to chemically couple the molecules to the substrate in a density and with appropriate linkers to result in enhanced growth rates of attached cells.

European Patent Application 531733

Claims 1-7, 9, 10, 13, 18-21, 23, 25, 26, 29-31 were rejected under 35 U.S.C. §102(b) as being anticipated by European Patent Application 531733 ("EP '733"). Claims 10-12 and 26-28 were rejected under 35 U.S.C. §103(a) over EP '733. These rejections are respectfully traversed if applied to the amended claims.

EP '733 discloses a carrier to which is immobilized a cell growth factor. The factor is attached to the carrier through a linker or spacer. The spacer is preferably about 2nm in length (page 3, line 53) and is a polymer compound such as polyethyleneimine, polyamino acid or polymethylene (page 3, lines 56-58). It is stated that the presence of a cationic group on the spacer molecule is desirable to aid in cell adsorption to the surface through electrostatic force (page 4, lines 1-3). EP '733 does not teach or suggest a cell growth factor "tethered" to a substrate as claimed by Applicants but rather discloses a cell growth factor "immobilized" on a substrate (see claim 1 and page 2, line 8).

The polymers that EP '733 teaches, for example, polyethyleneimine, are not soluble in aqueous solution and will bunch up in aqueous solution, making the spacers have an effective length of about 2 nm (see page 3, line 53). The polymers of the claimed compositions and methods, on the other hand, are very soluble in aqueous solution and will

extend to their full length, providing a wide range of movement (flexibility) to the factors attached thereto. This is a very important aspect of Applicants' tethers. As discussed in the application at page 6, lines 6-8 and 11-26 and page 7, lines 21-30, the tether must be flexible to allow the growth factor to contact the receptor and also to allow the growth factor-receptor complex mobility within the cell membrane. (See, for example, page 6, line 19, "Substantial mobility of a tethered growth factor is critical . . . .")

Moreover, the polymers used by Applicants do not bind to cells (PEO is, in fact, cell repellent), allowing even more freedom of movement for the bound growth factors and the factor-receptor complex. See the attached reference, Rempp et al., Polymer Preprints, ACS #220 (August 1991) which discusses the cell repellency of PEO. EP '733 teaches that the polymers used therein preferably include a cationic group, to aid in cell adsorption (page 4, lines 1-3). EP '733 thus teaches away from the Applicants' invention, because it teaches use of non water soluble polymers that will interact with the cell.

WO 89/05616

Claims 1-10, 12-26, 28, and 31 were rejected under 35 U.S.C. §102(b) as being anticipated by WO 89/05616 ("WO '616"). Claims 11 and 27 were rejected under 35 U.S.C. §103(a) over WO '616. These rejections are respectfully traversed if applied to the amended claims.

WO '616 discloses linear polymeric tethers having one end attached to a support and the second end attached to a biomolecule useful for cell culture. WO '616 discloses that the

tethers are heterobifunctional, having two reactive groups thereon, one which binds to the substrate and one which binds to the biomolecule. In other words, WO '616 does not teach or suggest that the tethers can bind more than one biomolecule, as claimed by Applicants. This is an important aspect of Applicants' claimed compositions and methods, as discussed in the application on page 7, lines 3-8 and page 12, lines 25-28. Applicants' tethers can bind more than one molecule of the same growth effector or can bind different growth effector molecules. Thus, the density of a growth effector molecule on a substrate can be increased without substantially increasing the number of cell-repellant tethers. Alternatively, for example, both insulin and EGF could be tethered to the same substrate, allowing presentation of both molecules to the cell.

Under the approach outlined in the WO '616, in theory any concentration of molecules could be attached. However, since only linear tethers, i.e. tethers with only one attachment site for a factor and one attachment site to the substrate, are used, going to lower concentration also increases the distance between factors and potentially inhibits the ability of receptor-factor complexes to interact in the cell membrane. Thus, at lower concentrations, signalling may not occur at all using linear tethers, because the factors are homogeneously spaced on the surface. By using a multi-functional tether, Applicants can go to very low factor concentrations and still allow receptor aggregation by virtue of having more than one factor on each tether. So, even though the tethers are perhaps very far apart (i.e. the distance from the center of one tether the center of the adjacent tether is more than 2X the

fully extended chain length of the tether), receptor-receptor interactions can still occur in the membrane after ligand-binding because the factors are locally clustered.

WO '616 does disclose use of tethers to attach biomolecules to support surfaces. Among the biomolecules mentioned are some of the growth effector molecules claimed by Applicants. However, the only examples in WO '616 involving cells involved tethering of collagen and fibronectin. WO '616 did not report enhanced growth of cells but only enhanced adhesion. These molecules are not "growth factors" but are rather cell adhesion promoters. Therefore, that the compositions and methods of WO '616 result in increased cell adhesion is not surprising.

EGF itself attenuates cell adhesion, in other words, cells may spread out on the substrate in the absence of EGF (i.e., they may adhere and spread on a substrate that bears a "high" tether concentration resulting in a "low" tether spacing, a spacing less than 2 times the radius of gyration), but round up (become less adherent) in its presence. Rounded cells generally do not undergo DNA synthesis in culture and in fact they may round up so much in the presence of a tethered factor that they don't adhere at all and undergo apoptosis (programmed cell death that occurs in the absence of sufficient adhesion). WO '616 does not teach how to tether EGF and get cell growth. WO '616 teaches the use of PEO (a polymer commonly grafted to surfaces to inhibit cell adhesion), and generally teaches high concentrations of tether with no specifics about how to cause cell adhesion in the presence of these types of tethers and avoid non-adherent cells. On the other hand, Applicants show how

to enhance cell growth by balancing use of polymeric water soluble tethers which do not bind to cells and the use of the proper amounts of tethered growth effector molecules. WO '616 teaches how to tether proteins, but not how to tether EGF so that cell growth will be enhanced.

WO '616 discloses that cell growth is achieved with the disclosed compositions with the addition of soluble growth effector molecules, in the form of serum. Applicants seek to add new claim 33 with this Amendment which includes the element that Applicants' composition functions to enhance cell growth without the addition of soluble growth effector molecules.

In summary, the claims have been amended to more clearly define novel and non-obvious aspects of the claimed compositions and methods. The cited prior art references do not teach or suggest compositions or methods for enhancing cell growth involving the use of a water soluble polymeric tether attached to a substrate and able to bind more than one growth effector molecules so that the molecules cannot be internalized by the cell and the growth of target cells is enhanced and wherein the cell do not bind to the tethers.

Allowance of all claims 1-6, 8-22, and 24-33, as amended, is earnestly solicited.

Respectfully submitted,

Collen A. Beard
Reg. No. 38,824

Dated: July 14, 1997

ARNALL GOLDEN & GREGORY
2800 One Atlantic Center
1201 West Peachtree Street
Atlanta, Georgia 30309-3450
(404) 873-8794
(4040) 873-8795 (fax)

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this Amendment along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Date: July 14, 1997

Collen A. Beard

APPENDIX: Claims as pending after entry of the Amendment

1. (twice amended) A composition for stimulating the growth of eukaryotic cells comprising

a biocompatible solid substrate,

biocompatible synthetic water soluble polymeric tethers, and

growth effector molecules,

wherein one end of each tether is covalently linked to the substrate and each growth effector molecule is covalently linked to a distal end of a tether so that the growth effector molecule cannot be internalized by cells attached to the substrate, and the growth effector molecules are attached to the substrate in a concentration effective to enhance the rate of target cell growth without internalization of the molecules, each tether is able to bind more than one growth effector molecule, and the cells do not bind to the tethers.

2. The composition of claim 1 wherein the form of the biocompatible substrate is selected from the group consisting of netting, individual and woven fibers, sponge and shaped polymers.

3. The composition of claim 2 wherein the shape of the shaped polymer is selected from the group consisting of dishes, bottles, solid particles, hollow particles, and polymers shaped to match a desired tissue shape.

4. The composition of claim 1 wherein the biocompatible substrate is selected from the group consisting of glasses, metals and biocompatible polymers.

5. (twice amended) The composition of claim 4 wherein the substrate polymer is selected from the group consisting of synthetic polymers and natural polymers.

6. (twice amended) The composition of claim 5 wherein the substrate polymer is selected from the group consisting of proteins, polysaccharides, [extracellular matrix proteins,] polyesters, polycapralactone, polyhydroxybutyrate, polyanhydrides, polyphosphazenes, polyorthoesters, polyurethanes, and combinations thereof.

8. (amended) The composition of claim 1 wherein the tether is selected from the group consisting of polyethylene oxide and carboxymethylcellulose.

9. The composition of claim 1 wherein the growth effector molecules are selected from the group consisting of epidermal growth factor, platelet-derived growth factor, transforming growth factor, hepatocyte growth factor, heparin binding factor, insulin-like

growth factor I or II, fibroblast growth factor, erythropoietin, nerve growth factor, bone morphogenic proteins, muscle morphogenic proteins, extracellular matrix molecules, and combinations thereof.

10. The composition of claim 1 wherein the tether has a backbone length between 5 and 50,000 atoms.

11. The composition of claim 10 wherein the tether has a backbone length between 100 and 50,000 atoms.

12. The composition of claim 10 wherein the tether has a backbone length between 5 and 500 atoms.

13. (twice amended) A method for growing eukaryotic cells comprising
bringing into contact the cells and a composition comprising
a biocompatible solid substrate,
biocompatible water soluble polymeric tethers, and
growth effector molecules,
wherein one end of each tether is covalently linked to the substrate and each growth effector molecule is covalently linked to a distal end of a tether so that the growth effector molecule cannot be internalized by cells attached to the substrate, and the growth effector molecules are attached to the substrate in a concentration effective to enhance the rate of target cell growth without internalization of the molecules, each tether is able to bind more than one growth effector molecule, and the cells do not bind to the tethers; and
maintaining the contacting cells and composition under conditions and for a time sufficient to cause the cells to grow.

14. The method of claim 13 wherein the step of bringing into contact comprises administering the composition to a patient in need of cell growth.

15. The method of claim 14 wherein the composition is administered by injection, infusion, or implantation.

16. The method of claim 15 wherein the composition is administered by implantation of the composition and wherein the substrate is shaped to match a desired tissue shape.

17. The method of claim 16 wherein the substrate is biodegradable.

18. The method of claim 13 wherein the form of the biocompatible substrate is selected from the group consisting of netting, individual and woven fibers, sponges and shaped polymers.

19. The method of claim 18 wherein the shape of the shaped polymer is selected from the group consisting of dishes, bottles, solid particles, hollow particles, and polymers shaped to match a desired tissue shape.

20. The method of claim 13 wherein the biocompatible substrate is selected from the group consisting of glasses and biocompatible polymers.

21. (amended) The method of claim 20 wherein the substrate polymer is selected from the group consisting of synthetic polymers and natural polymers.

22. (amended) The method of claim 21 wherein the substrate polymer is selected from the group consisting of polylactic acid, polyglycolic acid, polyanhydrides, polyorthoesters, collagen, glycosaminoglycans, polyamino acids, and combinations thereof.

24. (amended) The method of claim 13 wherein the tether is selected from the group consisting of polyethylene oxide, carboxymethylcellulose, and starch.

25. The method of claim 13 wherein the growth effector molecules are selected from the group consisting of epidermal growth factor, platelet-derived growth factor, transforming growth factor, hepatocyte growth factor, heparin binding factor, insulin-like growth factor I or II, fibroblast growth factor, erythropoietin, nerve growth factor, bone morphogenic proteins, muscle morphogenic proteins, extracellular matrix molecules, and combinations thereof.

26. The method of claim 13 wherein the tether has a backbone length between 5 and 50,000 atoms.

27. The method of claim 26 wherein the tether has a backbone length between 100 and 50,000 atoms.

28. The method of claim 13 wherein the tether has a backbone length between 5 and 500 atoms.

29. The method of claim 13 wherein the cells are selected from the group consisting of parenchymal cells and stem cells.

30. The method of claim 29 wherein the cells are hepatocytes.

31. (twice amended) A cell culture comprising
a biocompatible solid substrate,
biocompatible water soluble polymeric tethers,
growth effector molecules, and
growing cells,
wherein one end of each tether is covalently linked to the substrate and each growth effector molecule is covalently linked to a distal end of a tether so that the growth effector molecule cannot be internalized by cells attached to the substrate, and the growth effector molecules are attached to the substrate in a concentration effective to enhance the rate of target cell growth without internalization of the molecules, and wherein the growing cells are bound to the growth effector molecules and do not bind to the tethers and each tether is able to bind more than one growth effector molecule.

32. (twice amended) A method of testing a compound for an effect on tissue comprising
bringing into contact the compound to be tested and a composition comprising
a biocompatible solid substrate,
biocompatible water soluble polymeric tethers,
growth effector molecules, and
growing cells,
wherein one end of each tether is covalently linked to the substrate and each growth effector molecule is covalently linked to a distal end of a tether so that the growth effector molecule cannot be internalized by cells attached to the substrate, and the growth effector molecules are attached to the substrate in a concentration effective to enhance the rate of target cell growth without internalization of the molecules, and wherein the growing cells are bound to the growth effector molecules and do not bind to the tethers and each tether is able to bind more than one growth effector molecule;
incubating the compound and the composition under conditions promoting cell growth; and
observing the cells for any effect not observed in cells not brought into contact with the composition.

33. (new claim) A method for growing eukaryotic cells comprising
bringing into contact the cells and a composition comprising
a biocompatible solid substrate,
biocompatible water soluble polymeric tethers, and

growth effector molecules,

wherein one end of each tether is covalently linked to the substrate and each growth effector molecule is covalently linked to a distal end of a tether so that the growth effector molecule cannot be internalized by cells attached to the substrate, and the growth effector molecules are attached to the substrate in a concentration effective to enhance the rate of target cell growth without internalization of the molecules, each tether is able to bind more than one growth effector molecule, and the cells do not bind to the tethers; and

maintaining the contacting cells and composition under conditions and for a time sufficient to cause the cells to grow, without the addition of soluble growth effector molecules.